



Investigation of New Potential Uses of Menengiç (*Pistacia terebinthus*) for Various Areas

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ABSTRACT

Pistacia terebinthus has been used extensively in alternative medicine. The inhibitory activity of ethanol fruit extract from *P. terebinthus* fruit on food-borne and clinical test microorganisms was obtained to determine the potential use of the extract for food and pharmaceutical industries as a natural antimicrobial source. The antibacterial activity of the ethanol extract on fish-originated pathogen bacteria was also determined for its potential use in the feed industry. In addition, the effect of the extract on the probiotic bacteria originating from breast milk was tested to obtain the potential use of the extract together with probiotic bacteria for the pharmaceutical and food industries. Antimicrobial activity was obtained by performing micro-dilution and macro-dilution assays as well as disc diffusion. Among the food-borne and clinical test microorganisms, the highest inhibition zone diameter (22.39±1.92 mm) was detected on *Listeria monocytogenes*. The highest antibacterial activity on the fish pathogens was recorded as 17.67±0 mm against *Vibrio anguillarum* A4. It was determined that *P. terebinthus* fruit extract had higher antimicrobial activity against some test microorganisms than Amikacin and Gentamicin antibiotics. Antifungal activity on *Candida glabrata* was also investigated by counting viable cells. At a concentration of 20 mg mL⁻¹, no viable cells were determined after 24 hours. The extract inhibited all the tested LAB, however, with low MIC and MBC values. The sun protection factor (SPF) of the extract and the extract+cream mixture at 10 mL concentration was recorded as 9.36 and 7.51. The results of the study indicated that *P. terebinthus* fruit ethanol extract can be an alternative as a natural additive in various industries such as feed, food, pharmaceutical, and cosmetic industries.

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Menengiç (*Pistacia terebinthus*)'in Çeşitli Alanlar İçin Yeni Potansiyel Kullanımlarının Araştırılması

ÖZET

Pistacia terebinthus, alternatif tıpta yaygın olarak kullanılmaktadır. *P. terebinthus* meyvesinden elde edilen etanol meyve ekstresinin gıda kaynaklı ve klinik test mikroorganizmaları üzerindeki inhibitör aktivitesi, ekstrenin gıda ve ilaç endüstrilerinde doğal bir antimikrobiyal kaynağı olarak potansiyel kullanımını belirlemek için tespit edilmiştir. Etanol ekstresinin, balık orijinli patojen bakteriler üzerindeki antibakteriyel aktivitesi, yem endüstrisinde kullanım potansiyeli için belirlenmiştir. Ayrıca ekstrenin probiyotik bakterilerle birlikte ilaç ve gıda endüstrilerinde kullanım potansiyelini ortaya çıkarmak için ekstrenin anne sütü kaynaklı probiyotik bakteriler üzerindeki aktivitesi test edilmiştir. Antimikrobiyal aktivite, disk difüzyonunun yanı sıra mikro seyreltme ve makro seyreltme deneyleri yapılarak belirlenmiştir. Gıda kaynaklı ve klinik test mikroorganizmaları arasında en yüksek inhibisyon zon çapı (22,39±1,92 mm) *Listeria monocytogenes* üzerinde tespit edilmiştir. Balık patojenleri üzerinde en yüksek antibakteriyel aktivite *Vibrio anguillarum* A4'e karşı 17,67±0 mm olarak kaydedilmiştir. *P. terebinthus* meyve ekstresinin bazı test mikroorganizmalarına karşı Amikasin ve Gentamisin antibiyotiklerinden daha yüksek antimikrobiyal aktiviteye sahip olduğu belirlenmiştir. *Candida glabrata* üzerindeki antifungal aktivite, canlı

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hücre sayımı ile de araştırılmıştır. 20 mg mL⁻¹lik konsantrasyonda, 24 saat sonra hiçbir canlı hücre belirlenmemiştir. Ekstre, test edilen tüm LAB'leri düşük MİK ve MBK değerleri ile inhibe etmiştir. Ekstre ve ekstre+krem karışımının 10 mL konsantrasyonundaki güneş koruma faktörü (GKF) 9,36 ve 7,51 olarak kaydedilmiştir. Çalışmanın sonuçları, *P. terebinthus* meyve etanol ekstresinin yem, gıda, ilaç ve kozmetik endüstrileri gibi çeşitli endüstrilerde doğal katkı maddesi olarak alternatif olabileceğini göstermiştir.

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INTRODUCTION

Menengiç (*Pistacia terebinthus*) is an important plant species due to its biological activity and chemical properties (Köten, 2021). Menengiç (*Anacardiaceae*) is widely grown in the southern and western regions of Turkey (Özcan et al., 2020). In traditional remedies, menengiç fruits have been used in the treatment of many diseases such as eczema, throat infections, stomachache, rheumatism, asthma, and cough (Matthäus & Özcan, 2006). Recently, the medicinal use of compounds such as phenolic compounds, saponins, fatty acids, flavonoids, alkaloids, and terpenoids from *P. terebinthus* has gained attention (Rauf et al., 2017; Buriani et al., 2017; Özcan et al., 2020; Kaçar et al., 2022). *P. terebinthus* fruits have been involved in many studies due to their biological activities (Topçu et al., 2007; Naghmachi et al., 2022) and high oil content (Matthäus & Özcan, 2006).

Nowadays, clinical and food-borne microbial infections and gained antibiotic resistance are among the most important problems threatening the health of societies. Worldwide, microbial infections cause millions of deaths each year (Khameneh et al., 2019). The increased antibiotic resistance has led to a decrease in the effectiveness of antimicrobial drugs and even their ineffectiveness (WHO, 2014; Baym et al., 2016). Therefore, it is necessary to obtain new and natural antimicrobial agents from plants.

Aquaculture is one of the most important animal food sectors that can meet the protein needs of the World's population and compensate for the lack of food (Mabrok & Wahdan, 2018). The occurrence of infectious diseases in aquaculture causes important problems in the development of the sector and leads to significant economic losses (Direkbusarakom et al., 1998). Antibiotics are extensively used to treat diseases caused by microorganisms in aquaculture. The use of antibiotics adds to the extension of antibiotic-resistant bacteria and genes into other organisms (Watts et al., 2017). Antibiotic resistance has been reported in *Aeromonas hydrophila*, *V. anguillarum*, and *Yersinia ruckeri* as a result of antibiotics used in fish farms (Petersen et al., 2002).

Herbal antimicrobials can be natural alternatives to these antibiotics used in the prevention and treatment of bacterial infections in fish.

Probiotics are live microorganisms that benefit the host when taken in adequate amounts (Hill et al., 2014). Probiotic microorganisms have been very popular in recent years due to their multi-faceted health-promoting benefits such as anticancer, antidiabetic, stimulating the immune system, and anti-inflammatory (Song et al., 2015; Andrabi et al., 2016; Bhat et al., 2017). Plant extracts can be used to improve probiotic effects as natural and safety additives (Noor, 2017; Cocetta et al., 2019).

Ultraviolet radiation (UV) is divided into three regions UV-A (315-400 nm), UV-B (280-315 nm), and UV-C (100-280 nm) (Polonini et al., 2011). Exposure to UV radiation for a long time increases the risk of various skin diseases such as cancer and photoallergic reactions. Skin problems are mainly caused by UV-B (280-320 nm) radiation (Kim et al., 2022). The effectiveness of a sunscreen or sunscreen component to protect against UV-B radiation is measured by the sun protection factor (SPF) (Twilley et al., 2021). Sunscreens are chemicals that protect against the negative effects of the sun, especially UV radiation (Maske et al., 2013). Recently, natural substances have been recognized as potential sources due to their absorption of UV radiation and their sunscreen properties (Cefali et al., 2019; Al-Amoody et al. 2020; Kurzawa et al., 2022; Ibrahim et al., 2022).

In the present study, to determine the potential use of *P. terebinthus* fruit ethanol extract in various industries; (i) the antimicrobial activity of the extract on food-borne and clinical test microorganisms and fish-borne bacterial pathogens, (ii) the potential use of the extract together with the probiotic candidate lactic acid bacteria (LAB) originated from breast milk, and (iii) also SPF value of the ethanol extract and the ethanol extract+cream mixture were determined.

MATERIAL and METHOD

Preparation of Extracts

P. terebinthus fruits were purchased from a local

market in Adıyaman (Türkiye). The fruits were first washed under tap water to remove dust and then rinsed with pure water. After drying in an airy environment, the fruits were ground using a grinder (Waring, USA). The powdered fruit material (15 grams) was extracted with ethanol using the Soxhlet device for 24 hours. The sample was then filtered using the Whatman No. 1 paper. The extract was concentrated under a vacuum using a rotary evaporator (Heidolph, Germany) and stored in the dark at 4 °C until use. The crude extract was dissolved with dimethylsulfoxide (DMSO) and then sterilized by filtering through a 0.45 µm filter.

Determination of Antimicrobial Activity

The antimicrobial activity of *P. terebinthus* fruit ethanol extract was first determined by using a disc diffusion assay. Nutrient Broth (NB)-Agar (for *Escherichia coli* O157:H7, *E. coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853, *Bacillus cereus* RSKK 863, *Shigella sonnei* Mu:57, *Salmonella enteritidis* ATCC 13076, *Yersinia enterocolitica* ATCC 11175, *Micrococcus luteus* B-4375, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, *A. hydrophila* ATCC 19570), Yeast Extract Peptone Dextrose (YPD)-Agar (for *C. albicans* ATCC 10231, *C. glabrata* RSKK 04019), Tryptic Soy Broth (TSB)-Agar (for, *Lactococcus garvieae*, *Y. ruckeri*, *Streptococcus agalactiae*, *L. monocytogenes* ATCC 7644), Tryptic Soy Broth-NaCl (for *Vibrio alginoliticus*, *V. anguillarum* A4 and M1 strains), De Man, Rogosa and Sharpe (MRS)-Agar (for *Lactobacillus gasseri* MA-2, *L. gasseri* MA-3, *L. gasseri* MA-4, *L. gasseri* MA-5, *Lactobacillus fermentum* MA-7, *L. fermentum* MA-8, *Lactobacillus delbrueckii* MA-9, *Lactobacillus vaginalis* MA-10, *Lactobacillus plantarum* RSKK 1062) were used as growth medium. The test microorganisms (0.5 McFarland) were inoculated onto solid media. Sterile discs were placed on the agar medium and then 20 µL (2000 µg disc⁻¹) of *P. terebinthus* fruit extract was dropped onto the discs. Amikacin (AK, 10 µg disc⁻¹) and Gentamicin (CN, 10 µg disc⁻¹) antibiotics were used as control groups in the disc diffusion test. After incubation for 24 h, the inhibition zones were measured. The experiments were repeated in triplicate.

MICRO-dilution Method

MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bactericidal Concentration) or MFC (Minimum Fungicidal Concentration) values of the extract were obtained against the test microorganisms using a micro-dilution assay. The test microorganisms (0.5 McFarland) were added to the mixture of extract and growth medium and then vortexed. After incubation for 24 h, the non-growth concentration of the extract in the broth medium was obtained as MIC values. Then, the mixture was inoculated on the agar

media using spot dropping method. After the incubation period (24 h), the microbial growth-preventing extract concentrations on the agar media were recorded as MBC or MFC values.

Macro-dilution Method

Macro-dilution assay was also used to obtain the antimicrobial activity of the fruit ethanol extract against *C. glabrata* RSKK 04019 by counting viable cells using the method described by Sousa et al. (2006) with some modifications. *C. glabrata* RSKK 04019 suspension (0.5 McFarland) was added to the mixture of fruit ethanol extract (at concentrations of 5, 10, and 20 µg µL⁻¹) and growth medium. The cell suspension without the extract was used as a control group. Then, the control and test groups were incubated at 30 °C for 0, 24 and 48 h. The samples from the suspension were then diluted and inoculated onto YPD agar medium after each incubation time. The viable cell was counted and recorded as log₁₀ CFU mL⁻¹.

Determination of in-vitro Solar Protection Factor of Extract and Extract+Cream Mixture

The SPF value of *P. terebinthus* fruit ethanol extract was determined in vitro. The extract (2 µg µL⁻¹) was prepared in ethanol (96%) and measured in a spectrophotometer (Beckman Coulter) in the wavelength range of 290 nm-320 nm (at 5 nm intervals). The experiments were done in triplicate. The Mansur equation (1) used to calculate the sun protection factor was below (Mansur et al., 1986).

The SPF value of extract and cream mixtures was determined using the developed modified method (Asan Ozusaglam & Celik, 2023). 1 g of cream and 0.5 g of *P. terebinthus* fruit extract was mixed made up to 10 g with distilled water. The mixture diluted with ethanol to various concentrations (2.5 mL, 5 mL, and 10 mL) was measured using the spectrophotometer and then calculated as mentioned above.

Mansur equation:

$$\text{Solar protection factor (SPF)} = CF \sum_{\lambda=290}^{320} EE(\lambda) * I(\lambda) * Abs(\lambda) \quad (1)$$

Statistical Analysis

The mean values of the analysis results obtained in three replicates were evaluated statistically (IBM, SPSS Statistics 25 software, USA). One-way ANOVA and Tukey tests were used at the 5% level to make comparisons between means.

RESULTS and DISCUSSION

The antimicrobial activity of *P. terebinthus* fruit ethanol extract was investigated against food, clinical, and fish-originated test microorganisms using a disc diffusion assay. MIC and MBC or MFC values of the extract were also determined. In the current research, ethanol was used as the extraction solvent because the

ethanol solvent has good solubility of active substances compared to other solvents such as methanol, chloroform, and ethyl acetate (De Boer et al., 2005). The inhibition zone diameters of the extract on food-borne and clinical test microorganisms were determined between 22.39±1.92 mm and 10.03±0.06 mm. The results showed that the *P. terebinthus* fruit ethanol extract had antimicrobial activity on all test microorganisms tested. The amikacin and gentamicin antibiotics were used as the control group. It was

determined that the inhibition zone diameters of the fruit extract on food-borne and clinical microorganisms were generally higher than amikacin and gentamicin antibiotics. *P. terebinthus* fruit extract also showed an inhibition zone diameter of 10.42±0.35 mm and 17.67±0.26 mm on fish pathogens. It was obtained that the extract showed higher antimicrobial activity against fish pathogens *V. alginoliticus*, *V. anguillarum* M1, and A4 strains compared to the two commercially available antibiotics (AK and CN) (Table 1).

Table 1. Antimicrobial activity of *P. terebinthus* fruit ethanol extract
Çizelge 1. P. terebinthus meyve etanol ekstresinin antimikrobiyal aktivitesi

Test microorganisms	Inhibition zone diameter (mm)		
	Ethanol extract	AK	CN
Food-borne and clinical test microorganisms			
<i>E. coli</i> O157:H7	15.57±0.10 ^{a,h,I,s,u,w,z}	17.76±0	14.07±0.01
<i>E. coli</i> ATCC 35218	14.54±0.22 ^{c,h,I,q,s,u,z}	18.75±0.70	10.19±0.02
<i>L. monocytogenes</i> ATCC 7644	22.39±1.92 ^{e,h,I,q,s,u,z}	19.50±0.01	19.38±0.02
<i>B. cereus</i> RSKK 863	18.29±0.74 ^{b,d,f,g,k,m,o,q,w}	16.81±0.20	12.97±0.30
<i>S. sonnei</i> Mu:57	10.03±0.06 ^{b,d,f,I,k,q}	13.48±1.40	11.08±0.80
<i>S. enteritidis</i> ATCC 13076	12.75±0.88 ^{h,I,j,s,u,z}	13.87±0.01	10.51±0.02
<i>Y. enterocolitica</i> ATCC 11175	16.29±0.58 ^{h,l,q,z}	23.04±0.02	19.92±0.01
<i>M. luteus</i> B-4375	16.28±0.33 ^{h,n,q,z}	13.28±0.02	10.93±0.01
<i>P. aeruginosa</i> ATCC 27853	11.60±0.95 ^{d,f,h,I,m,o,p,s,u,w,z}	18.88±0.01	16.31±0.02
<i>S. aureus</i> ATCC 25923	15.13±2.09 ^{b,d,f,k,q,r}	17.34±0.01	13.05±0.02
<i>E. faecalis</i> ATCC 29212	17.29±0.35 ^{b,d,f,k,q,t}	24.02±0.30	13.48±1.44
<i>C. albicans</i> ATCC 10231	16.33±1.39 ^{b,h,q,v}	NA	NA
<i>C. glabrata</i> RSKK 04019	15.42±1.17 ^{b,d,f,k,m,o,q,y}	NA	NA
Fish pathogens			
<i>A. hydrophila</i> ATCC 19570	12.63±0.33 ^{a,f,h,I,k,m}	30.57±0.11	19.03±0.09
<i>Y. Rucker</i>	14.50±0.98 ^{c,f,h,I,k,m}	18.69±0.12	18.85±0.05
<i>V. anguillarum</i> M1	15.78±0.72 ^{b,d,e,h,k}	9.46 ±0.12	12.38±0.09
<i>V. alginoliticus</i>	16.25±0.24 ^{b,d,f,g,I,k,m}	15.03±0.03	15.06±0.07
<i>V. anguillarum</i> A4	17.67±0.26 ^{b,d,h,I,k}	12.07±0.13	15.13±0.15
<i>L. garvieae</i>	10.42±0.35 ^{b,d,f,h,I,j,m}	10.30±0.08	15.19±0.10
<i>S. agalactia</i> Pas.Ins. 55118	12.19±0.83 ^{b,d,h,k,l}	16.15±0.08	19.72±0.08

AK: Amikacin, CN: Gentamicin, NA: No activity

The different letters in the columns denote significant differences according to one-way ANOVA followed by Tukey's Multiple Comparison Test (P<0.05).

MIC, MBC, or MFC values of *P. terebinthus* fruit ethanol extract are given in Table 2. Among the food and clinical pathogens, the lowest MIC and MBC values of the extract were determined as 2.5 µg µL⁻¹ on *C. glabrata* RSKK 04019. The lowest MIC and MBC values (2.5 µg µL⁻¹) for fish pathogens were obtained on *V. alginoliticus* and *V. anguillarum* A4. Low MIC, MBC, and MFC values indicated that *P. terebinthus* fruit extract had high antimicrobial activity.

In a study, the antimicrobial activity of the methanol extract from *P. terebinthus* fruit collected from Elazığ (Turkey) was investigated by using a disc diffusion assay (Ereçevit & Kırbağ, 2017). In the study, the inhibition zone diameter of the methanol extract was recorded as 16 mm on *E. coli* and 17 mm on *S. aureus* which is close to the results of the current study. Çoban et al. (2017) investigated the antimicrobial activity of

the methanol, water, and ethyl acetate extracts from *P. terebinthus* fruit obtained from Aydın (Turkey) on various microorganisms. They reported the highest inhibition zone diameter on *E. coli* ATCC 35218 (13 mm) in fruit methanol extract and on *P. aeruginosa* (11 mm) in ethyl acetate extract. In the present study, nearly or slightly higher antimicrobial activity was obtained compared to the results of the study of Çoban et al. (2017). Plants secrete secondary metabolites as defense molecules, so factors such as the season in which the plant is harvested and the temperature of the environment can change the metabolites of the plant (Haliki et al., 2005). Therefore, the reason for the different results in biological activity may be due to the different environmental conditions, extraction methods as well as solvents used.

Table 2. MIC, MBC or MFC values of *P. terebinthus* fruit ethanol extract

Çizelge 2. *P. terebinthus* meyve etanol ekstresinin MİK, MBK veya MFK değerleri

Test microorganisms	Ethanol extract	
	MIC ($\mu\text{g } \mu\text{L}^{-1}$)	MBC or MFC ($\mu\text{g } \mu\text{L}^{-1}$)
Food-borne and clinical test microorganisms		
<i>E. coli</i> O157:H7	20	40
<i>E. coli</i> ATCC 35218	20	40
<i>L. monocytogenes</i> ATCC 7644	5	5
<i>B. cereus</i> RSKK 863	20	40
<i>S. sonnei</i> Mu:57	40	80
<i>S. enteritidis</i> ATCC 13076	40	40
<i>Y. enterocolitica</i> ATCC 11175	10	10
<i>M. luteus</i> B-4375	10	20
<i>P. aueruginosa</i> ATCC 27853	20	40
<i>S. aureus</i> ATCC 25923	5	5
<i>E. faecalis</i> ATCC 29212	40	80
<i>C. albicans</i> ATCC 10231	2.5	5
<i>C. glabrata</i> RSKK 04019	2.5	2.5
Fish pathogens		
<i>A. hydrophila</i> ATCC 19570	20	40
<i>Y. Rucker</i>	10	10
<i>V. anguillarum</i> M1	10	10
<i>V. alginoliticus</i>	2.5	2.5
<i>V. anguillarum</i> A4	2.5	2.5
<i>L. garvieae</i>	20	20
<i>S. agalactia</i> Pas.Ins. 55118	20	40

MIC (Minimum Inhibitory Concentration), MBC (Minimum Bactericidal Concentration), MFC (Minimum Fungicidal Concentration)

There are only a few antifungal agents in the treatment of *Candida* species, which are opportunistic human fungal pathogens, and one of the biggest problems is that they gain resistance to these antifungals in a short time (Silva et al., 2020). Therefore, the development of new natural drugs with fewer side effects for the prevention or treatment of fungal pathogens such as *C. glabrata* is of great interest. The antifungal activity of the extract on *C. glabrata* RSKK 04019 was recorded as 15.42 mm zone diameter in the disc diffusion assay. *P. terebinthus* fruit extract against *C. glabrata* RSKK 04019 was determined to have the lowest MIC and MFC values ($2.5 \mu\text{g } \mu\text{L}^{-1}$) among the foodborne and clinically tested microorganisms tested. Therefore, the antifungal activity of the fruit extract on *C. glabrata* RSKK 04019 was also investigated by counting viable cells and the results were given in Figure 1. At 5 and $10 \mu\text{g } \mu\text{L}^{-1}$ concentrations of *P. terebinthus* fruit extract, there was a certain decrease in viable cell count compared to the control group after 24 h. No viable cell was observed after 24 h and 48 h incubation at $20 \mu\text{g } \mu\text{L}^{-1}$ *P. terebinthus* fruit ethanol extract concentration.

Fungi infect billions of people each year but are still largely underappreciated as human pathogens (Brown et al, 2012). *C. albicans* and *C. glabrata* can be found particularly in the oral cavity and in the gastrointestinal tract of most healthy people (Fidel et

al., 1999; Cole et al., 1996). *C. glabrata* infections can be mucosal or systemic, and are more common in immunocompromised individuals or hosts with diabetes mellitus (Sinnott et al., 1987; Sobel, 1988; Geiger et al., 1995; Wingard, 1995). The antifungal activity of *P. terebinthus* fruit methanol extract collected from Elazig province on *C. glabrata* was investigated by Erecevit & Kırbağ (2017). In their study, they reported that the inhibition zone diameter of *P. terebinthus* fruit methanol extract on *C. glabrata* was 10 mm. A study showed that methanol, ethyl acetate, and boiled water extract obtained from *P. terebinthus* fruit had no antifungal activity against *C. glabrata* (Çoban et al., 2017). The higher antifungal activity obtained from this study may be due to the ethanol solvent used. Because ethanol has a better dissolving potential for active ingredients compared to other solvents.

LAB are microorganisms that are widely used as probiotic cultures in various processes that are generally considered safe (GRAS) and have therapeutic effects on the host (Gerez et al., 2013). The antimicrobial activity of *P. terebinthus* fruit extract against probiotic candidate LAB from breast milk, used at the concentration ($2000 \mu\text{g disc}^{-1}$) tested on pathogens is presented in Table 3. The results indicated that *P. terebinthus* fruit extract had inhibitory activity on all the tested LAB strains. The

disc diffusion assay results showed the lowest inhibition zone diameters as 11.91 ± 0.28 mm on *L. gasseri* MA-3 and 11.92 ± 0.37 mm on *L. fermentum* MA-2. The MIC and MBC values of the extract on the test LAB were determined as $10-40 \mu\text{g } \mu\text{L}^{-1}$ and $10-80 \mu\text{g } \mu\text{L}^{-1}$, respectively. The high MIC and MBC values of

the extract on LAB indicated that *P. terebinthus* fruit extract and LAB mixtures could have the potential to be used as natural preservatives in the food and pharmaceutical industries after adjusting the appropriate concentrations.

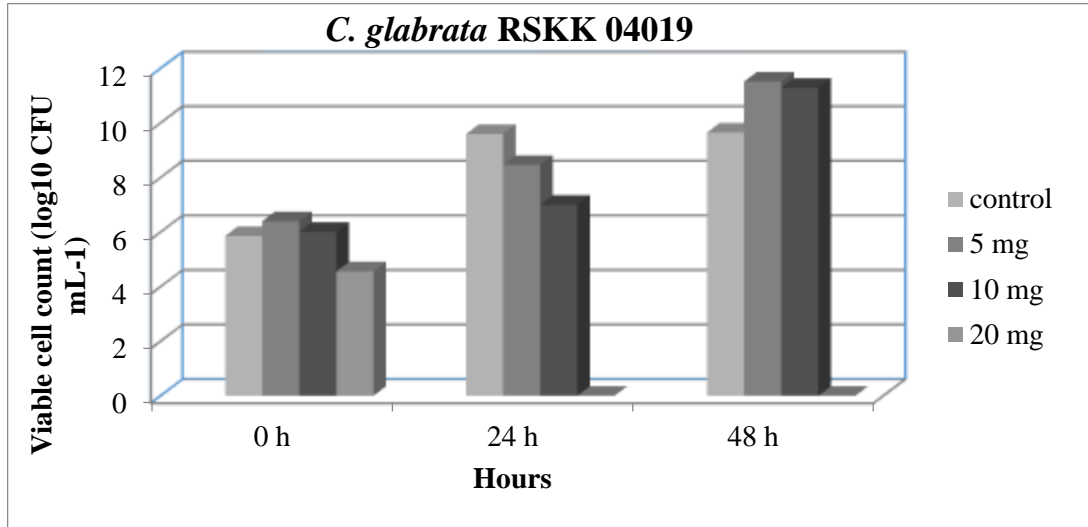


Figure 1. Antifungal activity of *P. terebinthus* fruit ethanol extract on *C. glabrata* RSKK 04019

Şekil 1. *P. terebinthus* meyve etanol ekstraktının *C. glabrata* RSKK 04019 üzerindeki antifungal aktivitesi

Table 3. Inhibitory activity of *P. terebinthus* fruit ethanol extract on probiotic candidate lactic acid bacteria originated from human milk

Çizelge 3. *P. terebinthus* meyve etanol ekstresinin insan sütü kaynaklı probiyotik aday laktik asit bakterileri üzerindeki inhibitör aktivitesi

Test microorganisms	Ethanol extract		
	Inhibition zone diameter (mm)	MIC ($\mu\text{g } \mu\text{L}^{-1}$)	MBC ($\mu\text{g } \mu\text{L}^{-1}$)
<i>L. gasseri</i> MA-2	11.92 ± 0.37	10	10
<i>L. gasseri</i> MA-3	11.91 ± 0.28	10	40
<i>L. gasseri</i> MA-4	13.18 ± 0.81	10	40
<i>L. gasseri</i> MA-5	$12.33 \pm 0.45^{\text{a,f}}$	10	20
<i>L. fermentum</i> MA-7	12.72 ± 0.05	40	80
<i>L. fermentum</i> MA-8	$11.95 \pm 0.58^{\text{c,f}}$	10	40
<i>L. delbrueckii</i> MA-9	$12.44 \pm 0.34^{\text{b,d,e}}$	10	40
<i>L. vaginalis</i> MA-10	12.82 ± 0.37	10	20
<i>L. plantarum</i> RSKK 1062	13.18 ± 0.03	20	80

MIC (Minimum Inhibitory Concentration), MBC (Minimum Bactericidal Concentration)

Values with the different superscript letters in the columns mean significantly different by one-way ANOVA followed by Tukey's post-hoc test ($P < 0.05$).

The antimicrobial activity of the *P. terebinthus* ethanol extract on the test microorganisms was statistically evaluated, and the results showed a statistically significant difference between food-borne and clinical test microorganisms ($P < 0.05$). The significant variation in inhibition zone diameter averages of the ethanol extract for fish pathogens or the LAB group was determined at a significance level of 0.05 ($P < 0.5$). Multiple comparison analysis using by Tukey test was performed to determine differences between means for each group and presented in Table 1 and Table 3.

The SPF value of *P. terebinthus* fruit ethanol extract was found to be 9.36. Sunscreens with an SPF of over 2 are considered to have good sun protective activity (Alves-Rodrigues & Shao, 2004). As a result of the literature review, no study was found showing the sunscreen effect of *P. terebinthus* fruit ethanol extract. The SPF value of the *P. terebinthus* fruit ethanol extract used in the presented study was found to be quite high.

The SPF values of the cream+*P. terebinthus* fruit ethanol extract mixture of various concentrations is

presented in Table 4. The SPF values of the cream+extract mixture were found higher than cream (control) at all tested concentrations. Therefore, it can be said that the addition of *P. terebinthus* fruit extract increased the SPF value of commercial cream. The

percentage of sun protection of *P. terebinthus* fruit ethanol extract at a concentration of 10 mL was found to be approximately 80% according to Imam et al. (2015).

Table 4. SPF values of *P. terebinthus* fruit ethanol extract+cream mixture

Çizelge 4. *P. terebinthus* meyve etanol ekstresi+krem karışımının SPF değerleri

Extract Concentration	SPF Values	
	Cream (Control)	Fruit Extract+Cream
2.5 mL	0.16	0.49
5 mL	0.47	1.03
10 mL	1.29	7.51

Recently, natural active compounds have been used in formulations to reduce the possible side effects of synthetic compounds and are accepted by consumers (Rodrigues et al., 2019). As a result of the literature review, no study was found to investigate the SPF value of cream mixtures of *P. terebinthus* fruit extracts.

CONCLUSION

The potential use of *P. terebinthus* fruit ethanol extract in various industries was investigated. The extract, with its good antimicrobial activity, can be used as a natural alternative for the food, feed, and pharmaceutical industries. Additionally, the extract and LAB mixtures, which combine antimicrobial activity and probiotic effects, can be used as natural additives in the pharmaceutical and food industries. In addition, the fruit ethanol extract of *P. terebinthus* showed good sun protection activity and even increased the SPF value of a commercial cream. Thus, the fruit ethanol extract could be an alternative as a natural additive for the cosmetic industry. *P. terebinthus* grows naturally in many regions but has limited use. This study revealed that *P. terebinthus* may be a new alternative for various industries and has the potential to be a natural resource to avoid synthetic additives.

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Conflict of interest

None.

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